both into the transition and intermediate states, and support our previous ideas of a 3(10)-helix region that moves in sequence in S4 in order to occupy the same position in space opposite F290 from the open through the three first closed states.

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What Hinges on the S3 Hinge?: Effects of a Conserved Proline Residue on Gating Current in S4-Based Voltage Sensors
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Voltage-gated potassium channels contain a highly conserved proline residue in their third transmembrane segment (S3). It has been proposed that the hinge, or kink, produced in the helix by this residue separates the S3 segment into an intracellular segment (S3a), and an extracellular segment (S3b). Mutating this proline to an alanine on the Shaker K^+ IR-H4, W434F background does not alter the gating current Q-V significantly, but it does result in a dramatic slowing of gating kinetics. As removal of prolines from transmembrane domains in other proteins is insufficient to remove the kink, we mutated a threonine, found four residues above the proline, and potentially involved in stabilizing the proline-induced kink. While the single mutation of the threonine to an alanine had little effect on the gating currents measured, the simultaneous mutation of the proline and threonine to alanine resulted in gating kinetics that were slower than wild-type, but faster than those obtained with the proline-alanine mutation alone. By inserting a proline into the homologous position of an inactivated (C363S), left-shifted (R217Q) version of Ci-VSP, we accelerated gating current kinetics. Displacement of the proline one residue in the extracellular direction slowed gating current kinetics and produced a rightward Q-V shift; conversion of this displaced proline to a serine also slowed gating currents compared to wild-type. These results suggest that the rapid gating kinetics promoted by the presence of the S3 proline are the result of the proline stabilizing a network of intramolecular bonds rather than by simply kinking the helix. Supported by NIH-GM030376.

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Molecular Basis for Time Dependent Modulation of Kv3.1 Channels that Assures Action Potential Repolarization
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Voltage-dependent potassium (Kv) channels constitute the cell's repolarizing power and shape the action potential (AP) duration. Excitable cells can choose from a large pool of Kv channels, each with different biophysical properties, and tune their AP shape by expressing a selected subset. Kv3 channels have been linked to high-frequency AP firing because of their high threshold of channel opening (around 0 mV) and fast closure kinetics during repolarizing potentials. However, although fast closure prevents the membrane from being excessively repolarized (affecting the next AP generation), it may result in incomplete membrane repolarization. Previously we determined that Shaker-type Kv1 channels display voltage-sensor relaxation, a process that results in slower channel closure. To investigate the relaxation process in Kv3.1, we recorded gating currents from Kv3.1 channels expressed in *Xenopus* Oocytes. In contrast to *Shaker*-type Kv1 channels, the relaxation process is in Kv3.1 very fast and the voltage-sensor gets stabilized in the ''up'' conformation even before channel opening. This behavior might originate in Kv3.1's particular gating kinetics that are characterized by a very steep voltage-dependency. Consequently, Kv3.1 channels actually close slowly (compared to channel opening) in the voltage range between −30 and 0 mV. Furthermore, upon short depolarizations they display a previously uncharacterized hooked tail current during subsequent membrane repolarization. This hooked tail was not linked to an underlying inactivated state and can be simulated with a kinetic model. A hooked tail current yields a temporal increase in repolarizing power that most likely secures membrane repolarization during the falling phase of the AP that normally works as a negative feedback mechanism on channel closure. Therefore, this time dependent modulation of Kv3.1 channel closure is expected to be physiologically important for high-frequency AP firing (Support: NIH-GM030376 and FWO-G043312.N)

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A Gating Modulator Peptide Toxin for *Shaker*-Type K_V1 Channels Derived from the Sea Anemone *Bunodosoma cangicum*
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Natural peptide toxins are a rich source of channel modulators that act on the extracellular face of K_v channels, either at the external pore mouth or at the voltage-sensing domain (S3-S4 linker). As such, natural toxins provide us with highly selective and potent molecular probes to unravel the structure and function of K_v channels. Toxins binding to the external pore mouth partly or completely block the K^+ permeation while those binding to the VSD inhibit channel function by modifying channel gating. The *Shaker*-related K_v1 channels are targeted by several peptide toxins that block the outer pore mouth but no gating modifiers that shift the voltage-dependence of channel opening have yet been identified. Furthermore, the $K_v1.5$ channel - an important target for the treatment of atrial fibrillation - has no known external peptide pore blockers, presumably due to the presence of a positively charged arginine residue in the outer pore mouth (R379, equivalent to *Shaker* T449). Bcg31.16 is a recently discovered peptide neurotoxin derived from the sea anemone *Bunodosoma cangicum* that inhibited several K_v1 subunits with nM potency. Bcg31.16 caused a concentration-dependent depolarizing shift in the voltage-dependence of channel opening; with 300 nM the shifts amounted to +35 mV and +12 mV for $K_v1.3$ and $K_v1.5$, respectively. The voltage-dependence of C-type inactivation displayed similar shifts, as well as the voltage-dependence of the gating kinetics. No significant effect on $K_v2.1$ was obtained at 1 microMolar. Thus, Bcg31.16 is a new gating modifier toxin of the K_v1 family and a novel peptide toxin to inhibit the $K_v1.5$ channel. (Support: FWO-G043312N to DJS and JT).

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1-Butanol and Gambierol:Low and High Affinity Compounds that Immobilize Charge Movement in *Shaker* and $K_V3.1$ Potassium Channels
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Voltage-gated K^+ (Kv) channels exist as tetramers of α-subunits that contain six transmembrane segments (S1-S6). The S5-S6 segments assemble into the ion permeation pathway that is surrounded by four voltage-sensing domains (VSD). Upon a membrane depolarization the VSD's move independently to the 'pre-activated state', and subsequently in a concerted way to the activated state that results in channel opening. It has been reported that 1-alkanols, such as 1-Butanol, inhibit Kv channels by binding to the S4-S5 linker and the bottom part of S6 (S6c), suggesting that they affect the gating machinery. Recently, we demonstrated that the polycyclic-ether toxin gambierol -that most likely binds to S6c outside the permeation pathway- impairs VSD movement in $K_v3.1$ channels. To determine whether 1-Butanol immobilizes the VSD similarly, we analyzed gating currents of *Shaker*-W434F channels expressed in HEK-293 cells. Determining WT *Shaker's* affinity for 1-Butanol based on the fraction of ionic current block yielded an IC_{50} value of ~50 mM. Concentrations that yielded full current block reduced charge movement in *Shaker*-W434F by at least 50%. Based on the loss in gating charge movement an IC_{50} value of ~260 mM was obtained which in a model with 4 identical binding sites fits the IC_{50} value of 50 mM obtained from ionic current inhibition. Kinetic analysis showed that 1-Butanol accelerated the deactivating gating currents similar to 4-AP indicating that the final concerted step could not be passed. However, this final concerted step carries only 5% of the total charge and 1-Butanol reduced charge movement by at least 50%. These data suggest that the mechanism of 1-Butanol is reminiscent to that of gambierol and stabilizes an early/deep closed state. (supported by fellowship CONACyT #176137 to EMM & grant FWO-G0433.12N to DJS)

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Stoichiometric Analysis of Heterotetrameric Kv2.1/Kv6.4 Channels
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Voltage-gated K^+ (Kv) channels are formed as tetramers of α-subunits. Based on sequence similarity, closely *Shaker*-related Kv subunits have been divided into eight subfamilies (Kv1-Kv6 and Kv8-Kv9). Members of the Kv1-Kv4 subfamilies form - within the subfamily - functional channels in both homo- and heterotetrameric configuration. The Kv5, Kv6, Kv8 and Kv9 subunits are designated silent Kv (KvS) subunits since they do not form functional homotetrameric channels, but they co-assemble with Kv2 subunits generating functional heterotetrameric Kv2/KvS channel complexes. Kv1-Kv4 channel subunits are thought to assemble as a dimer of dimers, but Kv2.1/Kv9.3 channels have been shown to assemble with a 3:1 stoichiometry - i.e. three Kv2.1 subunits with one Kv9.3 subunit. However,